

Malaria: Immunity and Prospects for Vaccination

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Malaria infections elicit a complex chain of cellular events which can, in some instances, lead to a state of immunity. Although there is strong evidence that a collaboration between specific antibodies and activated macrophages plays the central effector role in malaria immunity, alternative interpretations are possible. It is, for example, not known which malarial antigens are essential for triggering the critical effector functions and how these antigens are presented to the immune system. Under these circumstances, it is not surprising that the search for a vaccine against malaria has used rather empirical methods. Three invasive stages of the parasite (merozoites, sporozoites and gametes) have so far shown a potential efficiency in inducing protection in experimental models, but there is much to be done before vaccination can be an effective tool in malaria control.

MALARIA TODAY remains the most lethal of all human parasitic diseases. The global eradication measures, based upon the use of insecticides and chemotherapy, have greatly reduced its incidence, but administrative and financial problems, aggravated by the spread of insecticide resistant mosquitoes and drug resistant parasites, have reversed the gains in some areas, particularly in South East Asia. In Sri Lanka, for example, the incidence of malaria was reduced to less than 1,000 cases per annum in 1965, but had reverted to pre-eradication levels in 1976 (almost 1 million new cases reported).¹ With the realization that the present methods for malaria control are expensive and no longer adequate in some areas of the world, new tools are being sought and vaccination

against malaria, which was considered impractical by most scientists a decade ago, is now being reevaluated.

The general features of immunity to malaria have been extensively reviewed elsewhere²⁻⁶ and it appears that a variety of immune responses are elicited during the course of malaria infection: a great variety of antibodies are produced and there is a considerable stimulation of nonspecific reticuloendothelial functions. Nevertheless, the development of resistance in humans is so slow that in endemic areas many children may be intermittently ill for a number of years before any sign of resistance appears and many die before they have any resistance at all. The critical questions are the following: Why does protective immunity develop so slowly in natural infections? Which immune responses are protective and which are useless or even harmful? Could a vaccine achieve

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ABBREVIATIONS USED IN TEXT

ADCC=antibody-dependent cytotoxic T cells
 DNP=dinitrophenyl
 EE=exo-erythrocytic
 FCA=Freund's complete adjuvant
 HRP=histidine-rich polypeptide
 IMP=intramembranous particles
 LAF=lymphocyte-activating factor
 MDP=muramyl dipeptide
 MHC=major histocompatibility (antigen)
 MIF=migration inhibition factor
 NK=natural killer (cells)
 TNP=trinitrophenyl
 SRBC=sheep erythrocytes

protection more easily than the natural course of infection?

In this paper, I will consider the repertoire of immune responses that could be involved in promoting or inhibiting the development of resistance to malaria and examine the theoretical and practical problems that still remain to be solved before a vaccine against malaria can become a practical tool in the control of this disease.

Life Cycle of Plasmodium and Potential Points of Attack

There are four species that cause human malaria, *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, each with a characteristic course of infection and pattern of disease.⁷ However, most of the mortality associated with malaria is caused by *P. falciparum* and it is against this infection that a vaccine is most urgently needed.

Much of the present knowledge concerning malaria in man has been derived from field studies and from controlled infections in neurosyphilitic

patients and prisoner volunteers. Candidate vaccines, however, will have to be tested initially in animal models. Some New World monkeys—particularly owl monkeys (*Aotus trivirgatus*)⁸ and squirrel monkeys (*Saimiri sciureus*)⁹—are susceptible to *P. falciparum*, but these animals are limited in numbers and the course of infection differs from that in humans. Knowledge about malaria is derived largely from experimental studies carried out on laboratory animals infected with nonhuman malaria parasites. The organisms most commonly used for this purpose are *P. berghei*, *P. yoelii*, *P. vinckei* and *P. chabaudi*, which infect laboratory rodents, *P. gallinaceum* and *P. lophurae*, which infect chickens and ducks, and *P. knowlesi*, which infects rhesus monkeys.¹⁰ Each of these models presents specific advantages. It is worth mentioning here that none of these experimental models is a natural host-parasite combination (for example, *P. berghei* is a parasite of an African rodent, *Grammomys surdaster*, adapted to grow in laboratory rodents) and that the criticism of possible irrelevance, which is sometimes leveled at *P. falciparum* infection in nonhuman hosts, applies to all the common experimental models available.

The typical life cycle of mammalian *Plasmodium* sp is briefly the following (Figure 1): sporozoites are inoculated into the skin by infected anopheline mosquitoes and rapidly localize in the liver where they invade parenchymal cells. Inside these cells, each sporozoite develops into a schizont which will eventually liberate up to 20,000 individual merozoites capable of invading erythrocytes. Within the erythrocyte, the parasite divides and releases 6 to 20 merozoites which in turn can

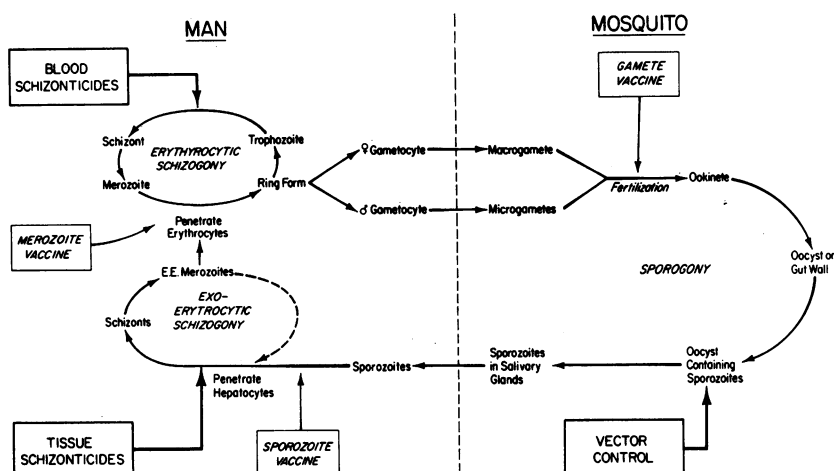


Figure 1.—Life cycle of malaria parasites showing the different points of attack either by classical control methods (chemotherapy and insecticides) or by vaccination.

invade new erythrocytes. In the course of this erythrocytic schizogony, a percentage of asexual parasites will develop into gametocytes which, when taken up by a mosquito, will transform into gametes. Fertilization occurs when a male microgamete penetrates into a female macrogamete. The ookinete that is produced migrates to the gut wall of the insect vector and develops into an oocyst. At the end of its development, each oocyst contains about 10,000 immature sporozoites, which mature and become infective in the process of their migration towards the salivary glands of the mosquito.

The malaria parasite is, therefore, intracellular during most of its life cycle, but is exposed to the extracellular space each time it invades a new host cell and during fertilization. The invasive forms are highly differentiated and equipped to invade only a specific cell type. Due to their extracellular nature, these invasive forms are the most obvious targets for an immunological attack (Figure 1). Once the parasite is intracellular, it is virtually invisible to the immune system and, given that it can resist the natural killing mechanisms of the host cell (which are negligible in erythrocytes), it cannot be recognized or destroyed by effector cells, unless the surface of the infected cell is modified by the parasite.

Critical Malarial Antigens

In the course of malaria infection, the immune system is exposed to a range of parasitic antigens, but, although their respective role in the induction of protection is unknown, some of these antigens could play a more critical role than others because of their location either on the surface of infected cells or invasive forms or their presence as soluble antigens in the bloodstream.

Surface of Invasive Forms

The surface of merozoites is covered by a thick filamentous coat which is morphologically similar to that described in trypanosomes and is believed to be involved in the recognition-adherence which will eventually lead to erythrocyte invasion; the coat appears to be shed from the surface when the merozoite enters into the red cell.^{11,12} The surface coat has been described as T-shaped bristles mounted perpendicularly to the cell membrane and has been shown to stain strongly with ethanolic phosphotungstic acid, to bind ruthenium red, alcian blue-lanthanum nitrate and cationized ferritin, and to be removed by proteases (criteria

which suggest a glycoprotein nature), but does not bind any of the usual lectins.^{13,14} The coat is present on the surface of merozoites before the mature schizont (segmenter) ruptures, but it becomes thicker after the merozoites are released into the circulation.¹⁵ By analogy with trypanosomes, some authors have suggested that the coat could consist of a single glycoprotein which would, as in trypanosomes,¹⁶ be involved in immune evasion by a process of antigenic variation,¹⁷ but this attractive hypothesis has not yet been confirmed experimentally.

In *P. knowlesi* infections, merozoites are agglutinated by immune serum in vitro and this effect is associated with reduced in vitro invasion.¹⁵ The inhibitory action is concentrated in the IgM and the IgG fractions of the immune serum—or the F(ab')₂ fragments—and is independent of complement.¹⁸ Although reinvasion inhibition is more pronounced with homologous than heterologous strains, it is not clear whether variant specific antibodies are directly involved. The fact that variant specific sera can be used to separate merozoites from schizont-infected cells by differential agglutination suggests that these antibodies do not take part in merozoite agglutination and in vitro invasion inhibition.¹⁹ Moreover, there appears to be a certain degree of correlation between clinical immunity and in vitro inhibition of invasion^{20,21} which further suggests that antibodies directed against surface components of merozoites may play a critical role in protection. Further, the binding of fluorescein-labeled antibodies to the surface of *P. berghei* merozoites in vitro has been shown to correlate with passive transfer of antibodies and protection.²²

Recent experiments using monoclonal antibodies have shown in *P. yoelii* that antibodies directed against the merozoite surface could be used to block the entry of the parasite into erythrocytes in vivo, whereas antibodies directed against other malarial antigens had no effect.²³ Little is known about the surface of exoerythrocytic (EE) merozoites (that is, merozoites released by liver schizonts) and whether these forms are identical to erythrocytic merozoites. In infections with the avian parasite *P. fallax*, a difference between these two forms has been shown to exist: when turkeys are infected with EE forms and treated with chloroquine to suppress erythrocytic development, their sera can be used to passively protect other turkeys against EE forms (that is, 90 percent of EE merozoites are de-

stroyed), but has no effect on the development of homologous erythrocytic stages.²⁴

The fact that the surface antigens of different invasive forms are stage-specific is not surprising, considering that they are equipped to recognize and invade such different cells. Antibodies against the surface of sporozoites can be detected by using the circum-sporozoite precipitation method²⁵: in presence of antibodies the formation of a precipitate around the terminal end of the sporozoite can be observed using phase-contrast microscopy. The surface of sporozoites not only differs antigenically from other stages of the same parasite, but also differs from that of sporozoites of unrelated species.²⁶ Furthermore, the surface antigens responsible for this precipitation change during sporozoite maturation: antiserum prepared against salivary gland forms does not cross-react with oocyst (immature) forms. When sporozoites of *P. berghei* are incubated in immune serum, they rapidly lose their infectivity. There is, however, little correlation between this in vitro sporozoite neutralizing activity and protection against sporozoite challenge.²⁷ The critical question so far as surface antigens are concerned is the following: does a single molecular species cover the entire organism or is there a mosaic of antigens? In the case of *P. berghei* sporozoites, a single protein, 44,000 MW, seems to account for all three biological activities: a monoclonal antibody directed against this protein (Pb44) produces a circum-sporozoite precipitation and in vitro neutralization, and can be used to passively protect against sporozoite challenge in mice.^{28,29}

Membrane-Associated Antigens

The membrane of malaria-infected erythrocytes undergoes dramatic changes as the parasite grows from the ring to the schizont stage. Surface labeling techniques have revealed both the loss of some components of normal membranes and the acquisition of others which were not present on the surface of normal cells.³⁰⁻³³ The appearance of parasite-specific components on the surface of infected erythrocytes is still controversial. *P. knowlesi* schizont-infected erythrocytes can be agglutinated by immune serum³⁴ and this agglutination has been shown to be strain-specific and submitted to a pattern of antigenic variation.³⁵ The variant antigens, however, have not yet been chemically defined and the evidence of their parasitic nature remains circumstantial. On the other hand, at least seven new proteins have been de-

tected by immunochemical methods on ghosts of infected erythrocytes prepared by nitrogen cavitation.³⁶⁻³⁹ There is no doubt about the parasitic nature of these proteins (they can be metabolically pulse-labeled, while erythrocyte components cannot), but their localization on the outer membrane of infected erythrocytes, rather than on the parasitophorous vacuole or the parasite membrane, has not yet been unambiguously demonstrated.

With *P. falciparum* and certain other primate malaria parasites, electron microscopic studies have shown the presence of dense areas protruding on the surface of infected erythrocytes,³⁹ which are believed to be responsible for the sequestration of schizont-infected cells in capillaries through adhesion to the endothelial epithelium. These structures, called knobs, may fail to appear on the host cell surface when the parasite has been submitted to long-term in vitro cultivation and knobless strains have been shown to lack a 80,000 MW protein.⁴¹ Knobs can induce an antibody response in immune animals⁴² and there is evidence suggesting that the immune recognition of surface determinants present on infected erythrocytes can be strain specific.⁴³ The hypothesis of knobs being parasitic material inserted into the erythrocyte membrane would be strengthened if a positive correlation among morphological, biochemical and immunological data were to be shown. Results of recent immunochemical studies have indicated that such a correlation might prove difficult to establish, in view of the instability and "stickiness" of the knob material.^{41,44}

In *P. berghei*, a parasite which preferentially invades reticulocytes, neither knobs nor variant-antigens have been detected on the surface of infected cells, but the binding of specific antibodies could be an indication of the existence of parasitic antigens on the surface of infected reticulocytes. Such binding has been visualized by immunofluorescence using monoclonal antibodies,²³ by immunoperoxidase staining⁴⁵ and by antitritonphenyl (anti-TNP) plaque formation.^{46,47} These results must, however, be interpreted with caution because infected reticulocytes have been shown to bind nonspecifically a variety of serum proteins⁴⁸; modified reticulocyte components, rather than parasite antigens, could be responsible both for the induction of specific antibodies and their binding.

No surface alteration which might suggest the presence of parasitic antigen has been described in malaria-infected hepatocytes. Such studies are

technically difficult: this stage has not yet been adequately maintained in vitro and, in vivo the percentage of infected cells is very low, even after a massive injection of infective sporozoites. Malarial antigens associated with the surface of cells harboring EE forms could, however, play an immunological role, particularly in parasite infection where there is a long-lasting exo-erythrocytic development (for example, *P. malariae*) or where EE forms are cyclic. The latter is the case in avian malaria, where EE forms occur in various cells and can be maintained in vitro (for example, in turkey brain cells).^{49,50}

The parasitophorous vacuole membrane is perhaps the membrane of erythrocytic origin most likely to carry membrane-associated malarial antigens because of its close contact with and its role in the metabolism of the malaria parasite. Its formation is induced by the release of rhoptry material from the apical end of the invading merozoite^{12,51} and it surrounds the parasite throughout its development. Shortly after its formation, this membrane has virtually no intramembranous particles (IMP)⁵² and histochemical studies of enzyme activity have shown that ATPase and NADH (nicotinamide adenine dinucleotide, reduced) oxidase activity were found on the same side as on the erythrocyte outer membrane, rather than being reversed as would be expected in an invaginated erythrocyte membrane.⁵³ When the parasite has reached the schizont stage, numerous IMP are found in the parasitophorous vacuole membrane,⁵² suggesting either that some parasite material has been inserted or that IMP from the outer erythrocyte membrane have moved to the interior, thus implying an intermittent continuity between these membranes, as well as a disruption of the spectrin network (spectrin does not normally allow lateral mobility of IMP). The immunological importance of the parasitophorous vacuole membrane is not known: this membrane is not exposed to the outside during the growth of the parasite, but is released in the form of vesicles at the time of schizogony. Other parasite material released in the circulation during the schizogony-reinvasion process includes the residual body (that is, digestive vacuole of the parasite containing the pigment) and the surface coat of merozoites. Most of this material is rapidly taken up by phagocytic cells and therefore could be involved with or interfere with the antigen presentation function of macrophages which, in the final analysis, represents the most efficient mechanism of recognition.

The recognition by lymphocytes of macrophage-processed malarial antigen has been shown in vitro.⁵⁴

Soluble Circulating Antigens

Soluble circulating antigens have been described in infections with *P. knowlesi*, *P. lophurae*, *P. galinaceum*, *P. berghei* and *P. falciparum*.⁵⁵⁻⁵⁹ The exact origin, nature and significance of these various antigens is not clear. In *P. falciparum* infections, heat-resistant soluble (S)-antigens have been described that are parasite strain specific—that is, the same S-antigens are always found in a given laboratory strain, whether it is maintained in an owl monkey, a squirrel monkey or in vitro.⁶⁰ This observation, in view of the variety of S-antigens found in clinical cases in a limited geographical area,⁶¹ suggests that the strain complexity of human malaria is much greater than is usually believed. Soluble antigens are usually considered to have a detrimental effect on the development of resistance against malaria: they bind specific antibodies and form circulating immune complexes, which can cause a range of immunopathological complications.⁶² Another possibility is that these soluble antigens are partially degraded malarial antigens (for example, a polysaccharide chain of a glycoprotein) which, although they are no longer immunogenic, can block the immune response by inactivating or removing specific antibodies from the circulation⁶³ or by inducing immune suppression.

Repertoire of Immune Responses to Malaria

The respective roles of the various components of the immune system in the development of an acquired immunity to malaria are still unclear, but all the available data point towards a complex mechanism involving both humoral and cell-mediated factors. What follows is an attempt to dissect this mechanism and examine how the different effectors might interact.

Innate Resistance and Natural Killer Cells

Malaria merozoites only can invade the erythrocytes of certain host species and within these species they usually have a preference for a given subpopulation of red cells. In the case of *P. knowlesi* and *P. vivax*, erythrocyte preference has been pinpointed to a defined erythrocyte receptor which is somehow associated with the presence of the Duffy blood group.⁶⁴ A second level of resistance occurs inside the erythrocyte where

cytoplasmic components can interfere with malarial growth: *P falciparum* has a preference for young erythrocytes⁶⁵ and it has been shown that erythrocytes containing certain abnormal hemoglobins are less favorable for its development.⁶⁶ In many circumstances where a genetically controlled resistance to infection has been detected, a linkage can be shown with a major histocompatibility (MHC) antigen. In *P falciparum* infections, such a linkage has so far not been found, although HLA-typing has been used in large scale epidemiological studies. However, a hint of evidence that that MHC genes may be important in malaria comes from a study of HLA phenotypes in four Sardinian villages: an excess of HLA-A₂ and HLA-B₁₇ genes was noted in the people in lowland villages, which have been exposed to malaria for the last two centuries, while no excess of any HLA genes could be found in the inhabitants of highland villages unexposed to malaria.⁶⁷ The higher frequency of antibody against malaria in these same two HLA phenotypes in Tanzania suggests that they are indeed associated with some resistance against malaria.⁶⁸

In rodent malaria, where experiments can now be carried out in inbred strains of mice having a precise H-2 background, all attempts to show a relationship between resistance and MHC factors have so far been disappointing. A variation in susceptibility of different strains of mice to rodent malaria does, however, exist⁶⁴ and it has recently been suggested that this variation could be related to a variable activity or levels of natural killer (NK) cells. Mice of strain A, which have a low NK activity, are highly susceptible to *P chabaudi* and do not have any great increase of NK activity (as measured by lysis of target tumor cells in vitro) at the peak of parasitemia, whereas strains which are less susceptible to this parasite (for example, CBA or C57B1) show a considerable increase of NK activity during malaria infection.⁶⁹ It is possible that NK cells are involved at a more specific level of resistance, particularly as these cells are stimulated by various substances including interferon. The latter has been shown to be present at high titers in the serum of malaria infected animals, probably under the influence of a high level of macrophage activation (see below). It could be suggested, in this context, that the nonspecific protection against *P berghei* in mice produced by the injection of BCG (bacillus Calmette-Guerin)⁷⁰ might be obtained through this pathway.

The Role of T Cells

There is abundant evidence that T cells play a role in the acquired immunity against malaria. In the case of rodent malaria, thymectomy of neonatal rats or treatment with antithymocyte serum causes a pronounced exacerbation of *P berghei* infection^{71,72} and a lethal infection with an otherwise mild strain of *P yoelii* develops in athymic nude mice.^{73,74} Resistance has been adoptively transferred with immune spleen cells,^{75,76} but little information is available on the transfer of purified cell populations. There has been no study of the kinetics of the different T lymphocyte subsets in the course of malaria infection, but an overall decrease of circulating T cells has been reported both in patients with *P falciparum*⁷⁷ and in rodent models⁷⁸ and seems to be related to a sequestration of these cells by the spleen.

The role of helper T cells has been explored by using parasites or parasitized cells as carrier for TNP and detecting the anti-TNP plaque forming response of the spleen at different stages of malaria infection or after immunization.⁴⁶ These studies showed little or no correlation between helper T cell response and protection against malaria suggesting that T cell functions other than the regulation of antibody formation are involved. The same is suggested by experiments using B-deficient mice which were drug-rescued from an otherwise lethal infection with *P yoelii*: these mice had a certain degree of resistance against the same strain despite the lack of detectable antibody.⁷⁹

Direct killing of parasitized cells by cytotoxic T cells has been reported in *P berghei* infections with an in vitro assay where the lysis of infected erythrocytes was shown by chromium 51 release,⁸⁰ but the interpretation of this result is difficult, particularly in view of the spontaneous fragility of mouse erythrocytes in vitro. Such killing could not be accomplished in a similar study using *P knowlesi*.⁸¹ Nevertheless, the increase in killer cell activity (associated with a general depletion of T cells and B cells) has been reported both in children with acute *P falciparum* infections⁸² and in rodent malaria models⁸³ and suggests that antibody-dependent cytotoxic T cells (ADCC) could participate in the protective response to malaria.

There is ample clinical and experimental evidence that acute malaria produces a temporary immunosuppression^{84,85} which might account for the greater severity of intercurrent infections (such as measles, respiratory infection and gas-

troenteritis) in children with malaria. The immune response to many antigens, including heterologous erythrocytes, human immunoglobulin, tetanus toxoid, *Salmonella typhimurium* and various murine viruses, is suppressed while the response to other antigens is normal (keyhole limpet hemocyanin is an example). The mechanism of this immunosuppression is not known, but there is little or no evidence to suggest a suppressor T cell involvement. In mice infected with *P. berghei*, there is an intense nonspecific immunosuppression which affects both T and B functions, but when spleen cells from infected mice are mixed with normal spleen cells there is no reduction of phytohemagglutinin (PHA)-induced transformation.⁶ Similarly, a normal response to PHA (1 gram per ml) was obtained with peripheral lymphocytes from children with acute falciparum malaria,⁸⁶ but with lower doses of PHA (0.1 gram per ml) a decreased response was noted, particularly in a case where chronic *P. malariae* infection was associated.⁸⁷ On the other hand, the peripheral blood lymphocytes of *Aotus trivirgatus* showed a reduced response to various mitogens at the acute stage of *P. falciparum* infection⁸⁸ and, when the serum of such animals was added to normal owl monkey or human lymphocytes, a decreased mitogen response was also observed.⁸⁹ An inhibitor of lymphocyte proliferation could not, however, be found in sera of patients with falciparum malaria.⁹⁰

The function of T cells in promoting macrophage activation through the secretion of lymphokines has not been examined thoroughly in malaria. In *P. yoelii* infected mice the study of macrophage kinetics suggests that monocytes leave the bone marrow and accumulate in the liver and spleen just before recovery.⁶ This migration occurs more rapidly in specifically vaccinated mice and these newly arrived macrophages appear to be highly activated but it is not known whether they actually destroy the parasites. The specific accumulation of monocytes in the spleen seems to be under the influence of a 80,000 MW chemotactic factor secreted by the spleen cells throughout malaria infection and which can be detected as early as four hours after infection—that is, about 48 hours before any detectable increase of splenic macrophages.⁹¹ Moreover, the role of T cells in the recruitment and activation of macrophages has been demonstrated in T deficient (nu/nu) mice, which show little splenomegaly and no enhancement of phagocytosis during malaria infection, whereas B deficient mice behave normally.⁹²

The disappearance of macrophages from the peritoneal cavity and the detection of a macrophage migration inhibition factor (MIF) in spleen cells of acutely infected mice points in the same direction.⁹³ Delayed hypersensitivity, as measured by the homing of labeled bone marrow cells to the ear after local challenge with the antigen—that is, *P. yoelii*-infected red cells—is greatly increased in specifically vaccinated mice, particularly in conditions which lead to protection.⁹⁴ These results suggest the existence of a T cell function with protective value, but, at the same time, they indicate that T cells alone are not responsible for protection.

The Role of Macrophages

The role of macrophages in malaria was recognized 40 years ago by pathologists, who showed an increased number of macrophages in the spleen, liver and bone marrow of infected animals. During the parasitic crisis (when parasitemia starts to decrease under the influence of acquired immunity) there is a considerable increase in phagocytosis of malaria parasites, infected erythrocytes and malarial debris (particularly pigment).⁹⁵ In rodent malaria, where the phagocytic activity of splenic macrophages can be 20- to 50-fold greater than that of normal macrophages,⁴ there is an increased uptake of normal as well as infected red cells.⁹⁶ This increase in phagocytic activity has been linked both to a specific activation of macrophages and to humoral factors, particularly opsonizing antibodies, which act synergistically with macrophages to enhance the destruction of the parasites.⁹⁷⁻⁹⁹ The enhancement of phagocytic activity, however, does not always last throughout the infection and can, for unknown reasons, become normal or subnormal before there is any reduction in the level of parasitemia, suggesting that, at this stage of infection, phagocytic activity is no longer indispensable for the development of functional immunity.¹⁰⁰

Activation of macrophages produces a variety of substances which can trigger other immune functions. Among these, interferon production has been demonstrated,¹⁰¹ and the presence of interferon has been related to resistance, at least against sporozoite infection¹⁰²; the role of interferon in increasing NK activity has been suggested as a possible effector mechanism.⁶⁹ In this context, the function of macrophages in immunity has recently been analyzed with agents known to inhibit or stimulate macrophage activity in vivo (silica

and *Corynebacterium parvum*, respectively): in normal mice, administration of silica accelerated the appearance of parasites in the blood and *C parvum* delayed it, whereas in vaccinated mice, the clearance of parasitemia was accelerated by silica and delayed by *C parvum*. These results suggest that macrophages, especially if activated, could inhibit the development of sterilizing immunity, apparently by inhibition of the IgG response.¹⁰³ This paradoxical effect might be related to a reduced secretion of lymphocyte-activating factor (LAF)¹⁰⁴ and the production of suppressor factors by adherent spleen cells of malaria-infected mice.¹⁰⁵ The helper function of macrophages appears to be normal at the beginning of infection, but is severely altered later.^{106,107} This has been shown in a study of in vitro antibody formation in response to sheep erythrocytes (SRBC) and dinitrophenyl (DNP)-Ficoll by adherent spleen cells from infected and normal mice; between days 2 and 4 of a *P yoelii* infection, the antibody response to both antigens was enhanced and a pronounced reduction was observed, between days 8 and 21, only in the case of SRBC, while the response to DNP-Ficoll (which is macrophage-independent) was normal.¹⁰⁷

The Role of Antibodies

The role of antibodies in resistance against malaria has been studied by three different approaches: the passive transfer of immune sera, the in vitro effect of immune serum on isolated parasites and the infection of B deficient animals.

Passive transfer experiments show that antibodies merely cause a transient reduction of the parasitemia.¹⁰⁸⁻¹¹² This reduction can be sufficient to allow the host to develop an effective response and thus recover from an otherwise fatal infection. In *P falciparum* infections, large amounts of IgG from immune adults (doses equivalent to 10 percent to 20 percent of the recipient's own IgG) reduced the parasitemia to undetectable levels in heavily infected children,¹⁰⁹ but this protection was of short duration. The relative resistance of infants in hyperendemic areas could be explained by the passive transfer of antibodies from the mother, although other factors, such as the presence of fetal hemoglobin or the age of erythrocytes¹¹³ could be involved. The variability observed in passive transfer experiments, particularly in rodent malaria, seems to be related to the doses of immune serum administered as well as the variable quality of these sera. The fact that

large amounts of serum from recently recovered mice does not confer any protection against *P yoelii*¹¹⁴ might be due to the persistence in convalescent serum (as opposed to hyperimmune serum) of soluble malarial antigens and immunosuppressive factors, the role of which has been discussed above. The protective effect of monoclonal antibodies directed against the surface components of merozoites or sporozoites of *P berghei*^{23,29} clearly supports such a view. The activity of hyperimmune serum could partially be related to opsonization,^{99,115} particularly as passive serum transfer is more effective in intact than in splenectomized recipients.¹¹⁶⁻¹¹⁷ In *P knowlesi* infections, when antiserum against a given variant population is transferred to an animal infected with this variant, a transient reduction of parasitemia is obtained and a new variant population is induced, which, being no longer controlled by the antiserum, produces a lethal infection.¹¹⁸ Moreover, variant-specific antibodies have different kinetics than the antibodies responsible for opsonization, which suggests that they represent different entities.¹¹⁹ The transfer of T depleted immune spleen cells confers the same degree of protection to *P berghei* as spleen cells not T depleted, suggesting that B cells alone were responsible for the protective effect.¹²⁰ However, contradictory results from similar experiments¹²¹ and the large body of data concerning the involvement of T cells do not favor such an interpretation.

It has been reported that immune sera can affect sporozoites, merozoites and gametes in vitro. The sporozoite neutralizing test and the circum-sporozoite test can be used to demonstrate ant sporozoite activity of immune sera. The correlation of these assays with protection is generally considered to be poor,²⁷ although the results obtained with monoclonal antibodies suggest that this poor correlation might be related to other factors.²⁸ The effect of immune serum on merozoites can be measured with an in vitro reinvasion assay and inhibitory activity as has been demonstrated with *P falciparum* and *P knowlesi*. In the latter case, the sera from immune animals inhibit reinvasion by producing an agglutination of merozoites^{15,18}: this assay was found to correlate well with the results of transfer experiments,¹²² but not always with functional immunity.¹²³

An interesting observation has been made by using frozen blood samples taken from patients infected with *P falciparum* in The Gambia. In vitro reinvasion was tested in the presence of

homologous sera (that is, sera taken from the same patient at later stages of infection or after recovery) and heterologous sera. Inhibitory titers were always higher with homologous sera, probably in connection with the above-mentioned strain complexity of *P. falciparum*.¹²⁴ The sera from patients who have recovered from falciparum malaria have no effect on either bloodstream gametocytes¹⁰⁹ or on the infectivity of these sexual forms for the mosquito vector.¹²⁵ In some simian malaria, however, a striking contrast between the high number of gametocytes present in the bloodstream on day 14 of infection and the failure to produce any infection in the vector, has been reported.¹²⁶ Furthermore, in chickens immunized against gametes of *P. gallinaceum*, the infectivity of gametes can be completely suppressed in vivo, and in vitro the immune serum has been shown to block fertilization and ookinete formation. Two types of interactions between immune serum and gametes have been described: a surface fixation reaction, where small numbers of gametes are loosely attached together, and an agglutination reaction, where large numbers of gametes are clumped. A correlation between these in vitro assays and the suppression of infectivity was observed only in the case of the agglutination reaction.¹²⁷ With monoclonal antibodies directed against surface antigens of gametes, a surface fixation reaction can be observed, but agglutination (and suppression of infectivity) is only obtained by mixing monoclonal antibodies against two different surface components.¹²⁸ The use of B deficient animals, whether bursectomized chickens¹²⁹ or mice rendered deficient by treatment from birth with anti- μ serum,^{74,79} gives further evidence of the role of antibodies in acquired immunity to malaria, and shows that a collaboration between T cells and B cells is necessary for maximum protection.

Another aspect of the antibody response in malaria infections is that considerably more IgG is produced than can be accounted for by specific antimalarial antibodies.¹³⁰ It has been shown that a polyclonal stimulation by malaria mitogens could, at least to some extent, explain this effect.¹³¹ The nonspecific polyclonal B cell activation, which occurs during the course of malaria infections, does not result from a direct stimulation of B lymphocytes by mitogenic factors but has been shown to be T dependent. The following chain of events has been proposed: T cell mitogens, which are produced either by the parasite or by

the macrophages of infected animals, activate T cells, which in turn contribute to a B cell activation through a T helper effect^{17,132}; the detailed mechanisms of this phenomenon are not yet fully understood.

The Role of Complement

A depletion of various complement components, particularly C3, has been reported to occur during the course of infection by various malaria parasites.¹³³⁻¹³⁶ In the synchronous *P. knowlesi* infection, the reduction of complement activity paralleled the peak of schizogony and was therefore attributed to the release of free parasites into the bloodstream.¹³⁷ On the other hand, the peak of schizogony corresponds to the maximum release of circulating antigens and therefore of the formation of immune complexes. It could be argued that the solubilization of immune complexes, a function of the alternative pathway, might be responsible for the decreased level of complement. Interestingly, both the presence of immune complexes and of the complement cleavage product C3b are known to activate macrophages and could, thus, play a role in triggering the chain of events that leads to protection. The nonspecific protection against malaria conferred by *C. parvum*, an agent that activates the complement alternative pathway, could be related to this mechanism.¹³⁸ No direct complement-mediated cytotoxicity has been reported.

Overall Interactions

Assuming that the results obtained in the various experimental systems can be generalized, the following chain of events is suggested to explain the development of a functional immunity in malaria (Figure 2): (1) the recognition of the various malarial antigens (that is, surface of invasive forms, host-cell membrane associated antigens, soluble circulating antigens and antigens presented by macrophages) involves different types of T cells (for example, helper T cells are more likely to recognize malarial antigen associated with Ia antigens on the surface of macrophages rather than free antigen, while suppressor T cells are more likely to recognize free antigen); (2) these T cells, in turn, activate various effector cells, particularly B cells and macrophages; (3) the products of these effector cells will either react directly with the parasite (for example, invasion inhibition by antibodies or through the effect of interferon on host cells) or can act synergistically

with another effector cell or product to cause a cytotoxic effect (for example, opsonization by macrophages or ADCC) or can stimulate other functions (for example, interferon activation of NK cells), and (4) this chain of events is regulated in various ways by the parasite: circulating malaria antigens bind antibodies (thereby reducing serum antibody levels against the parasites). Soluble antigens also form circulating immune complexes, which in turn activate complement pathways and macrophages. Activated macrophages produce various factors, some of which interact with T cell functions and secondarily influence antibody formation. During the acute phase of infection, suppressive factors are produced that interfere with macrophage antigen-processing functions and cause negative feedback inhibition of T cell functions. At the same time, mitogens are secreted which *sidetrack* the immune system into a polyclonal B cell activation, probably through a T helper effect. Immunopathological side effects can be produced by many of these mechanisms, but their role in protection is not known.

Which Vaccine?

In view of the complexity of the immune response induced by natural malarial infections, it is possible to imagine that effective protection could be obtained more easily by a mechanism that stimulates only one kind of effector cell and,

therefore, bypasses all the detrimental regulatory factors. The choice of immunogen is obviously critical but, in the present state of our knowledge, it is not possible to decide which particular antigen, if any, would make the best vaccine. The current views of malaria vaccination^{3,24,138} are the following: (1) the best protection is obtained by a low level infection which will lead to a state of premunition; (2) only live, attenuated parasites (such as irradiated sporozoites) can produce complete protection; (3) killed parasites are generally only efficient in the presence of adjuvants and, at best, only achieve a partial stage-specific protection, which allows the host to survive a mild initial infection and, thus, built-up premunition; (4) parasite extracts or semipurified parasite extracts are generally ineffective even in the presence of adjuvants; (5) purified malarial antigens might succeed where parasite extracts have failed; (6) an interruption of the life cycle by interaction of invasion-blocking antibodies with invasive forms is generally considered to be the most hopeful line of investigation; finally, (7) interruption of transmission can be achieved without protecting the immunized recipient (for example, gamete vaccine).

The search for a malarial antigen that can induce an effective immunity has recently taken two different directions: the purification of stage-specific antigens and the preparation of antibodies with specific inhibitory activity. The purification

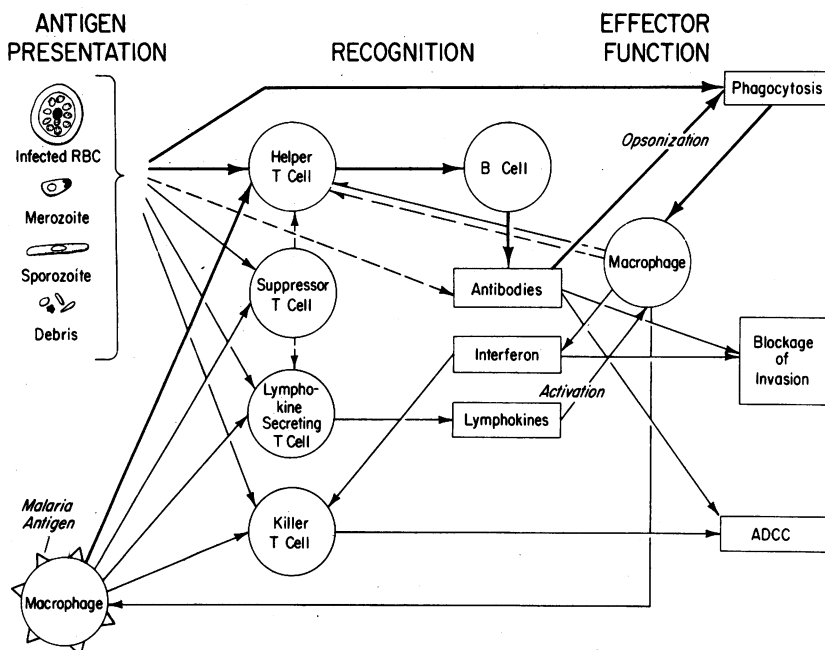


Figure 2.—Overall interactions of the different components of the immune system in response to malaria infection.

ADCC = antibody-dependent cytotoxic T cells, RBC = red blood cell (erythrocyte)

of stage-specific parasite populations is usually necessary before stage-specific antigens can be isolated. Merozoites can be separated from blood cells by using different methods: agglutination with lectins or antibodies,^{139,140} filtration through a small pore-size cell-sieve,¹⁴¹ elution from affinity columns^{142,143} or centrifugation of in vitro culture supernatants.¹⁴⁴ None of these methods is perfect and the merozoite preparations obtained are always somewhat contaminated with host cell material. Nevertheless, such merozoite preparations have been used for vaccination experiments with the following results: merozoites of *P knowlesi*, when given with Freund's complete adjuvant (FCA), gave a partial protection against challenge with erythrocytic stages of homologous and heterologous strains of *P knowlesi*^{140,145} or against a sporozoite challenge¹⁴⁶; the results obtained with other adjuvants (Freund's incomplete adjuvant with or without muramyl dipeptide [MDP] derivatives, saponin) gave less consistent results.¹⁴⁷ Merozoites of *P falciparum* have been used to partially protect owl monkeys in conjunction with either FCA¹⁴² or MDP derivatives.^{148,149} In rodent malaria, most vaccination experiments using merozoites have given disappointing results.³

The purification of sporozoites requires the dissection of infected mosquito salivary glands followed by the separation of sporozoites and mosquito material by gradient centrifugation^{150,152} or differential elution from lectin columns.²⁸ The contamination with mosquito material is generally considered to be less critical than the contamination with blood cell components, particularly as some kind of additional nonspecific protection can be conferred by mosquito extracts alone¹⁵³; such sporozoite preparations have actually been used in preliminary clinical trials. Most of the work on sporozoite vaccination has been done in the rodent models, where irradiated sporozoites have been shown to produce a complete, sterile immunity in more than 90 percent of the immunized animals.²⁵ Moreover, sporozoite vaccines do not appear to require any adjuvants. Sporozoite vaccination in simian models^{154,155} or in human volunteers^{156,157} yields more variable results and is generally less efficient. The protection conferred by sporozoite vaccines is usually of short duration: three months or less.

Gametocytes can be purified by differential centrifugation¹⁵⁸ and gametes can be prepared by controlled gametogenesis in vitro followed by differential centrifugation to separate the extra-

cellular gametes from blood cells and asexual parasites. Gamete vaccines have been explored in *P knowlesi*, *P gallinaceum* and *P berghei* with similar results: the vector infectivity of the blood of immunized animals could be totally suppressed by circulating antibodies. These antibodies are taken up in the blood meal and interact with the gametes as soon as they become extracellular in the mosquito gut and, thereby, prevent fertilization.¹⁵⁹⁻¹⁶¹

A purified histidine-rich polypeptide (HRP), isolated from *P lophurae*-infected erythrocytes, and believed to represent rho-try material,¹⁶² has been used without any adjuvant to immunize ducks. A partial protection was obtained in most immunized animals—that is, a mild infection developed with an otherwise lethal parasite.¹⁶³ In this case, no preliminary separation of parasites from host cell material was necessary because of the peculiar chemical nature of this antigen (soluble in acetic acid). Unfortunately, this exciting result (the only circumstance where a purified antigen has protected against malaria) could not be reproduced in another laboratory using similar experimental conditions and somewhat larger groups of animals¹⁶⁴ and this obviously calls for further experimental data.

All these experiments suffer from one major drawback: the amount of parasite material necessary for immunization is considerable and the methods available for the production of such material are generally inadequate. Erythrocytic forms of *P falciparum* can be grown in vitro in fresh or outdated human red blood cell cultures¹⁶⁵ but the parasitemia obtained does not exceed 10 percent and parasites are usually asynchronous. Techniques have been developed to concentrate parasitized cells,^{166,167} to obtain short-term synchronous cultures¹⁶⁸ and to induce the transformation of asexual stages into gametocytes,¹⁶⁹ but their yield is generally low. Parasite material obtained from infected experimental animals (for example, owl monkeys) or from human placenta in hyperendemic areas¹⁷⁰ has been used in preliminary studies, but cannot be considered as a suitable source of antigenic material for human vaccination. The situation is worse in the case of sporozoites because the sporogony cannot yet be achieved in vitro and salivary glands of infected mosquitoes still remain the exclusive source of parasite material. The case of histidine-rich protein, a potentially efficient malarial immunogen, is a good example of the present limitations of

malarial vaccines: 100 ml of infected duck blood containing approximately 100 percent schizont-infected cells (*P. lophurae* produces synchronous infections) is necessary to prepare 2 ml of free parasites, which corresponds to 25 mg of HRP¹⁷¹ and 2 mg of HRP is required to immunize a single duckling.¹⁶³ If the same material were to be prepared from *P. falciparum* cultures, assuming that the cultures contain 10 percent asynchronous parasites and that *P. falciparum* contains as much HRP as *P. lophurae*, at least 6 liters of human blood, 3 liters of human serum and 30 liters of medium would be required to produce 25 mg of HRP. Obviously, the solution lies elsewhere than in the direct purification of antigens from malaria parasites. The DNA recombinant technology could, for example, be of value if the antigen desired for vaccination proved to be a short polypeptide chain. Chemical synthesis could be envisaged if the critical antigen were carbohydrate in nature.

The second approach for the recognition of vaccinating antigens is the use of specific antibodies with an inhibitory activity against one of the three invasive forms. This approach, which essentially uses the hybridoma technology for the production of monoclonal antibodies, has recently given encouraging results with all three invasive forms in rodent and in avian models^{22,27,128} and should lead rapidly to the isolation of the relevant antigens. Furthermore, the possible use of anti-idiotypic serum directed against such protective monoclonal antibodies could represent an alternative to vaccination with malarial antigens and bypass the difficult problems of antigen production.

Finally, two types of interaction between pharmacological agents and the immune system have to be considered. On the one hand, the treatment with antimalarial drugs can either impair the development of immunity by reducing the duration of antigen stimulation¹⁷² or favor the development of immunity by reducing the level of parasitemia and, therefore, producing a more protracted course of infection. On the other hand, a variety of compounds can be used as immune adjuvants and all vaccination procedures using killed parasites have been shown to require some kind of adjuvant.¹⁷³ The role of these adjuvants is imperfectly known and it is likely that with a better understanding of the effectors of malaria immunity such empirical additives will no longer be required.

Conclusion

There is now a strong body of experimental data to suggest that malaria vaccines could provide a more solid immunity than the natural infection and could therefore prove to be a powerful tool in the fight against malaria. There is still a long way to go before trials of malaria vaccines can be envisaged in man; the problems in need of solution include the recognition and purification of suitable malarial antigens, the development of reliable in vitro tests to measure protective immunity and a better knowledge of strain complexity and possible cross-reactions between different strains. Moreover, if an effective vaccine against malaria were to become available, its application in the developing world would require massive international support and collaboration. Indeed, many of the available vaccines against infectious diseases are still not extensively used in the areas where they are most needed, because the affected nations cannot afford their purchase and do not have the medical infrastructure for their distribution.

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